COMPLIANCE ENGINEERING

545 E. Main Street • P. O. Box 149 • Hartsville, TN 37074-0149
(615) 374-4745 • Fax: (615) 374-0306

August 20, 2014

TN DEPT OF ENVIRONMENT
AND CONSERVATION

AUG 21 2014

DIV OF WATER RESOURCES
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TN Division of Water Pollution Control
William R. Snodgrass Tennessee Tower
312 Rosa L. Parks Avenue, 11th Floor
Nashville, TN 37243

Ref: Cothron's Grocery
Facility ID#4-560096
TNG830204

Dear Sir:

Please find enclosed the Toxicity Identification Evaluation/Toxicity Reduction Evaluation (TIE/TRE) Study for the above referenced site. This study was required as a result of two consecutive failures of the IC25 Wet Testing.

Sincerely,

Mark D. Harper, P.E.

Cc:
Mr. Wm. Oakley Hall
Environmental Field Office Manager
Division of Water Resources
Cookeville Environmental Field Office
1221 South Willow Avenue
Cookeville, TN 38506

Mr. Elwin (Rocky) Hannah - Cookeville EAC
1221 South Willow Avenue
Cookeville, TN 38506

Test Type: Acute or Chronic? Chronic
Screen or Definitive? N/A (TIE)

TOXICITY TEST REPORT SHEET - *Ceriodaphnia* TIE

1). Facility/Discharger:	Compliance Engineering- Cothron's Grocery — Report Date: 8/6/2014
2). Address:	P.O. Box 7, Hartsville, TN 37074
3). KPDES Permit #:	TNG830204 — 4). Receiving Stream: West Fork Long Creek
5). Facility Contact:	Mr. Mark Harper — 6). Phone #: (615) 374-4745 epharper@aol.com; ejpllc@aol.com
7). Consultant/Testing Lab Name:	ESC Lab Sciences
8) Lab Contact:	Shain W. Schmitt — 9). Phone #: (615) 773-7549
10). Outfall(s) Tested:	(TIE) Final Effluent samples from original test date: June 24- July 1, 2014 (L706249-01,-02,-03)

Average daily flow on days sampled (MGD):

Sample #1	Sample #2	Sample #3
not on C-of-C	not on C-of-C	not on C-of-C

11). Test Species:	*Ceriodaphnia dubia*
12). Species Age:	Neonates, <24-hr
13). Organism Source:	ESC Lab Sciences
14). Acclimation Procedure:	Cultured in Reconstituted Synthetic Freshwater at 25 deg C
15). Test Conditions: (Static or Static-Renewal?)	Static-Renewal
16). Dilution Water Type (synthetic, receiving stream):	Reconstituted Synthetic Freshwater
17). Aeration? (Before/During Test):	none, except during Aeration Test
18). Dechlorination?	none
19). ESC Lab Sciences Sample #:	TIE sample manipulations (L708706-01 thru -13)

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Signature of person filling out form — 8-18-14 (Date)

Shain W. Schmitt (Name (typed or printed)) — Sr. Aquatic Biologist (Title)

ESC Sample #: L708706-01 thru -13

Sampling Summary

Outfall	Type: Grab or Composite	Volume Collected	Sample Collection Begin MM/DD/Time	Sample Collection End MM/DD/Time	Rain Event?
	*composite	4 gallons		6/23/2014 @ 13:00	
	*composite	4 gallons		6/25/2014 @12:30	
	*composite	6 gallons		6/27/2014 @ 12:00	

Comments: *The samples above were originally used in a chronic biomonitoring test June 24- July 1, 2014. Because toxicity was demonstrated during the evaluation, TIE testing was initiated July 9, 2014 using remaining sample from the June 24- July 1, 2014 event.

Date/Times of Test Performance

Species #1

Ceriodaphnia dubia
(name)

7/9/2014 @ 16:20 to 7/16/2014 @ 09:24

Species #2

(name)

Baseline	-01
EDTA 3 mg/L	-02
EDTA 8 mg/L	-03
Sodium Thiosulfate (STS) 10 mg/L	-04
Sodium Thiosulfate (STS) 25 mg/L	-05
Aeration	-06
Granulated Activated Carbon (GAC)	-07
pH 3 Adjustment/Filtration	-08
pH 11 Adjustment/Filtration	-09
Zeolite	-10
Filtration	-11
C-18 SPE Column	-12
UV	-13

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Toxicity Test Results

Results of a *Ceriodaphnia* (Genus) *dubia* (Species) 3-Brood Survival & Reproduction (Type/Duration) (for TIE purposes)

Conducted _____ to _____

Using Effluent from Outfall: Comp Eng- Cothron's Grocery

-01 Test Solution	Percent Surviving - *Ceriodaphnia* (time intervals used - days)								BASELINE TEST (composite) # of Young		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	IC25 result:
100% Effluent	100	100	90	90	90	90	90	90	5	0.5	**25.4%**

-02, -03 Test Solution	Number of Live *Ceriodaphnia* (time intervals used - days)								EDTA Test (composite) # of Young		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	IC25 result:
EDTA 3 mg/L	100	100	100	100	100	100	90	90	31	3.1	**27.4%**
EDTA 8 mg/L	100	100	100	100	100	100	100	100	35	3.5	**27.8%**

-04, -05 Test Solution	Number of Live *Ceriodaphnia* (time intervals used - days)								Sodium Thiosulfate Test (composite) # of Young		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	IC25 result:
STS 10 mg/L	100	100	100	100	100	90	90	90	15	1.5	**26.1%**
STS 25 mg/L	100	100	90	90	90	90	90	90	12	1.2	**25.9%**

-06 Test Solution	Number of Live *Ceriodaphnia*								Aeration Test (composite)		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	
Aeration Contro	100	100	100	100	100	100	100	100	384	38.4	IC25 result:
100% Effluent	100	100	90	90	90	90	80	80	9	0.9	**25.7%**

-07 Test Solution	Number of Live *Ceriodaphnia* (time intervals used - days)								GAC Test (composite) # of Young		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	
GAC Control	100	100	100	100	100	100	90	90	67	6.7	IC25 result:
GAC	100	100	100	100	100	100	100	90	10	1.0	**25.7%**

-08 Test Solution	Number of Live *Ceriodaphnia* (time intervals used - days)								pH 3 Adj/Filt Test (composite) # of Young		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	
pH 3 Control	100	100	100	100	100	100	80	80	191	19.1	IC25 result:
100% Effluent	100	100	100	100	100	100	100	100	34	3.4	**27.7%**

Comp Eng- Cothron's Grocery ESC TIE Lab ID: L708706-01 thru -13

Toxicity Test Results

Results of a *Ceriodaphnia* (Genus) *dubia* (Species) 3-Brood Survival & Reproduction (Type/Duration) (for TIE purposes)

Conducted _____ **to** _____

Using Effluent from Outfall: Comp Eng- Cothron's Grocery

-09 Test Solution	Number of Live *Ceriodaphnia* (time intervals used - days)								pH 11 Adj/Filt Test (composite) # of Young		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	
pH 11 Control	100	100	100	100	100	90	90	90	285	28.5	IC25 result:
100% Effluent	100	100	100	100	100	100	100	100	322	32.2	**> 100%**

-10 Test Solution	Number of Live *Ceriodaphnia* (time intervals used - days)								Zeolite Test (composite) # of Young		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	
Zeolite Control	100	100	100	100	100	100	100	100	321	32.1	IC25 result:
Zeolite	100	100	100	100	100	100	100	100	375	37.5	**>100%**

-11 Test Solution	Number of Live *Ceriodaphnia* (time intervals used - days)								Filtration Test (composite) # of Young		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	
Filtration Contro	100	100	100	100	100	100	100*	100*	352*	39.1*	IC25 result:
Filtration	100	100	90	90	90	90	90	90	27	2.7	**27.1%**

*Daphnid 'Replicate J' was marked as NT (not transferred) at 144 hrs. The count for Total Young was divided by 9 instead of 10 to arrive at the mean (per EPA methods).

-12 Test Solution	Number of Live *Ceriodaphnia* (time intervals used - days)								SPE C-18 Test (composite) # of Young		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	
C-18 Control	100	100	100	100	100	100	100	100	350	35.0	IC25 result:
100% Effluent	100	100	90	90	90	90	90	90	25	2.5	**26.9%**

-13 Test Solution	Number of Live *Ceriodaphnia* (time intervals used - days)								UV Test (composite) # of Young		
	0	1	2	3	4	5	6	7	Total	Mean	
Control	100	100	90	90	90	90	80	80	351	35.1	
UV Control	100	100	100	100	100	100	100	100	365	36.5	IC25 result:
UV	100	100	90	90	80	70	50	50	0	0.0	**25.0%**

Scope of Work

A Toxicity Identification Evaluation (TIE) systematically attempts to characterize, identify, and confirm the causative agents of whole effluent toxicity. A TIE can help identify the class of compounds that are the cause of toxicity and then develop a strategy to reduce that class of compounds from the effluent.

A typical TIE scheme involves subjecting wastewater samples from suspect waste streams to a series of characterization tests. Each characterization test is designed to alter or render biologically unavailable a group of potentially toxic compound(s) such as oxidants, cationic metals, volatiles, nonpolar organics, or metal chelates. Aquatic toxicity tests are conducted on the wastewater aliquots of untreated and treated effluent to reveal whether the treatments were successful in reducing toxicity and to provide information on the nature of the toxicant(s). After a consistent pattern is observed in repeated TIEs, analytical chemistry procedures are used (Phase II) to identify specific toxicant(s) from the group(s) characterized in the previous step (Phase I). The final step in a TIE scheme consists of a group of steps intended to confirm the identity of the suspected toxicant(s) and establish the true cause of toxicity. For this test event (January 22-28, 2014), remaining sample from the Jan 7-14, 2014 evaluation was used to run the following tests (100% effluent with each): Baseline, EDTA (3mg/L & 8mg/L), Sodium Thiosulfate (10mg/L & 25mg/L), Aeration, Granulated Activated Carbon (GAC), pH 3 Adjustment/Filtration, pH 11 Adjustment-Filtration, Zeolite, Filtration, C-18 SPE Column, and UV treatment to characterize toxicity.

Brief descriptions are given in the Interpretation of Results section of this report. For more information, refer to U.S. Environmental Protection Agency's (U.S. EPA's) *Methods for Aquatic Toxicity Identification Evaluations: Phase I: Toxicity Characterization Procedures*, EPA-600/3-88/034 September 1988.

General Comments on Effluent Variability

Because effluent can vary in chemical composition over a period of time, results from one set of TIEs performed on samples collected from one point in time may not provide sufficient information about the facility's effluent toxicity problem. Furthermore, it is possible that different toxicants are responsible for the toxicity or aggravate the toxicity of the final effluent at different times of the year (for example, toxicity may be associated with monthly housekeeping procedures). Toxicity may also vary because of treatment plant efficiency, and treatment plant efficiency may vary with the time of day or season.

The issue of effluent variability can be particularly challenging with so many contributing factors, therefore more than one complete Phase I toxicant characterization series is recommended. The exact number of tests to be conducted is facility-specific. Consistent characterization results of a number of effluent samples are needed prior to moving to Phase II toxicant identification procedures.

Interpretation of Results: Baseline

The **Baseline Test** is the focal point of the Phase I portion of the TIE. The Baseline tests the permittee's unmanipulated wastewater. Survival and reproduction of the organisms in this treatment are compared to all post-treatment bioassay tests. If toxicity (expressed in terms of the concentration of effluent that causes a 25% reduction in survival or reproduction) is decreased in any of the characterization tests (as compared to the baseline test), then that group of compounds would be a suspected causative agent(s) of toxicity in that sample.

***Ceriodaphnia dubia* (water flea):**

Toxicity remained persistent in the *Ceriodaphnia* Baseline test. Using Linear Interpolation Method, the IC25 (inhibition concentration that will cause a 25% reduction in survival or reproduction of the test organisms) was determined to be 25.4% effluent (reproduction). The IC25 result for the Baseline Test (25.4% effluent) is the result that will be compared to all other treatments used during this particular TIE series.

It is important to note that the effluent continued to have a toxic effect on the *Ceriodaphnia* (with regards to reproduction) even after the sample had been held for up to a week from the initial toxicity evaluation June 24 - July 1, 2014. This is noteworthy because it is an indicator that the toxicant(s) remain persistent over time.

Interpretation of Results: EDTA 3mg/L and 8mg/L

Ethylenediamine Tetraacetate Chelation (EDTA) Test: 3mg/L and 8mg/L

If toxicity is attributable to cationic metals (other than anions such as selenium, arsenic, and chromate), the addition of a chelating agent, ethylenediamine tetraacetate (EDTA), may render them biologically unavailable. One stock solution of EDTA was prepared and used in two separate treatments during the test: a 3mg/L test and an 8mg/L test. Results of the 3mg/L and 8mg/L EDTA tests were compared to the Baseline test to determine whether the EDTA helped to remove or reduce toxicity.

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***Ceriodaphnia dubia* (water flea):**

Toxicity (with regards to reproduction) was still present after the addition of EDTA. In the samples treated with EDTA (3mg/L & 8mg/L), the daphnids continued to exhibit inhibition with regards to reproduction. Using Linear Interpolation Method, the IC25 (inhibition concentration that will cause a 25% reduction in survival or reproduction of the organisms) was determined to be 27.4% effluent for the EDTA (3 mg/L) and 27.8% effluent for the EDTA (8 mg/L). The EDTA did not prove effective in removing or reducing toxicity in the effluent sample, therefore toxicant(s) in this particular sample are not likely to be cationic metals.

Interpretation of Results: STS 10mg/L and 25mg/L

Oxidant Reduction Test (addition of sodium thiosulfate, 10mg/L and 25mg/L)

Sodium thiosulfate, a strong reducing agent, was added to the effluent sample to determine if oxidants such as chlorine, bromine, iodine, ozone, chlorine dioxide, and chloramines were contributing to effluent toxicity. A series of two increasing amounts of sodium thiosulfate were added to effluent samples to arrive at an appropriate concentration to reduce toxicants (but not enough to cause reagent-induced toxicity). One stock solution of sodium thiosulfate was prepared and used in the treatments: a 10mg/L STS treatment and a 25mg/L treatment. Following the addition of the STS to the effluent samples, the toxicity tests were conducted with *Ceriodaphnia* .

Interpretation of Results: STS 10mg/L and 25mg/L

***Ceriodaphnia dubia* (water flea):**

Toxicity (with regards to reproduction) was still present after the addition of STS. In the samples treated with STS (10mg/L & 25mg/L), the daphnids continued to exhibit inhibition with regards to reproduction. Using Linear Interpolation Method, the IC25 (inhibition concentration that will cause a 25% reduction in reproduction of the organisms) was determined to be 26.1% effluent for the STS (10 mg/L) and 25.9% effluent for the STS (25 mg/L). With no reduction in toxicity exhibited, the toxicant(s) are not likely to be an oxidant (like chlorine, bromine, iodine, chlorine dioxide, or chloramine).

Interpretation of Results: Aeration

Aeration Test

Changes in toxicity due to aeration of the effluent sample may be caused by substances that are oxidizable, spargeable, or sublatable. If the aerated effluent is less toxic than the Baseline test, the results would indicate that aeration is an effective removal technique. If the effluent toxicity is not reduced (or it is more toxic after aeration than in the baseline test), then toxicity was concentrated during the aeration process.

***Ceriodaphnia dubia* (water flea):**

Toxicity (with regards to reproduction) was still present after Aeration. Using Linear Interpolation Method, the IC25 (inhibition concentration that will cause 25% reduction in survival or reproduction of the test organisms) was determined to be 25.7% effluent.

Interpretation of Results: Granulated Activated Carbon

Granulated Activated Carbon (GAC)

Granulated activated carbon (GAC) is a highly porous absorbent material commonly used for dechlorination, organic chemical reduction and radon reduction, and is recognized by the USEPA as the best available technology for the reduction of organic chemicals from drinking water.

***Ceriodaphnia dubia* (water flea):**

Toxicity remained evident in the Granulated Activated Carbon (GAC) treated sample. Using Linear Interpolation Method, the IC25 (inhibition concentration that will cause 25% reduction in survival or reproduction of the test organisms) was calculated to be 25.7% effluent.

Interpretation of Results: pH Adjustment/Filtration

pH Adjustment/Filtration

A portion of the effluent was adjusted to pH 3, and another portion to pH 11 (and held at those levels for a period of one hour). Often, precipitation occurs with drastic pH changes. If precipitation does occur, then the filtration and pH adjustment test would likely remove the toxicant(s). The pH adjusted samples were filtered through a one (1) micron filter, and the pH was adjusted back to pH_i (initial pH of the effluent sample) prior to the *Ceriodaphnia* being loaded into the test vessels.

***Ceriodaphnia dubia* (water flea):**

Toxicity was still present after the pH 3 Adjustment/Filtration treatment, however, the pH 11 Adjustment/Filtration treatment proved effective in removing the toxic effects of the effluent sample. The IC25 (concentration of effluent that causes a 25% reduction in survival or reproduction of the test organisms) of the pH 3 Adjustment/Filtration sample was calculated to be 27.7% effluent, but the IC25 of the pH 11 Adjustment/ Filtration sample was determined to be greater than 100% effluent. Baseline sample produced an average of 0.5 neonates per adult. The pH 11 Adjustment/Filtration sample produced an average of 32.2 neonates per adult.

Interpretation of Results: Zeolite

Zeolite

Zeolite treatment is recommended in *Toxicity Reduction Evaluation Protocol for Municipal Wastewater Treatment Plants* (EPA/600 2-88/062 April 1989) as a means to identify ammonia toxicity: "Aeration in conjunction with pH adjustment is used to evaluate toxicants with volatility, such as ammonia or hydrogen sulfide."

After being rinsed in DI water, 90 grams of Zeolite was allowed to soak for a period of four (4) hours in Baseline effluent. Zeolite granules were removed after the soak period and test organisms were loaded.

***Ceriodaphnia dubia* (water flea):**

Zeolite proved effective in removing the toxic effects of the effluent sample. Using Linear Interpolation Method, the IC25 (inhibition concentration that will cause a 25% reduction in survival or reproduction of the test organisms) was determined to be greater than 100% effluent. Baseline sample produced an average of 0.5 neonates per adult. Daphnids in the Zeolite sample produced an average of 37.5 neonates per adult.

Interpretation of Results: Filtration

Filtration

The filtration step is designed to determine whether toxicity is in the suspended particulate phase or in the soluable fraction. The effluent is filtered through a one micron membrane filter to remove particulate.

***Ceriodaphnia dubia* (water flea):**

Toxicity remained evident in the Filtration treated sample. Using Linear Interpolation Method, the IC25 (inhibition concentration that will cause a 25% reduction in survival or reproduction of the test organisms) was determined to be 27.1% effluent (in reference, the Baseline IC25 = 27.4% effluent).

Interpretation of Results: C18 SPE Column Test

C18 SPE Column

The C18 SPE column is used to determine the extent of an effluent's toxicity that is due to compounds that are absorbed onto the column. Compounds extracted by the C18 column include primarily non-polar organics, but may also include some metals, and some surfactants. In addition, the columns may also behave as a filter. The effluent that passes over the column is collected and the post-column effluent is tested in order to determine if the column removed the toxicity.

***Ceriodaphnia dubia* (water flea):**

Toxicity remained evident in the C-18 solid phase extraction sample. Using Linear Interpolation Method, the IC25 (inhibition concentration that will cause 25% reduction in survival or reproduction of the test organisms) was determined to be 26.9% effluent (in reference, the Baseline IC25 = 27.4% effluent).

Interpretation of Results: UV Treatment

UV Treatment

Pathogenic organisms in effluent samples may affect test organism survival and confound test results. UV treatment has been shown, in some cases, to minimize pathogen interference. It must be noted, however, that UV exposure may have other effects on the sample. UV treatment has the potential of increasing or decreasing toxicity from other pollutants in the sample (beyond the beneficial treatment of the pathogens). UV treatment is known to cause photoactivation of organic compounds, which may increase toxicity. UV treatment is also known to cause the photochemical breakdown of organic compounds, which could decrease toxicity. These effects should be considered in the interpretation of the UV treatment data.

***Ceriodaphnia dubia* (water flea):**

Toxicity remained evident in the UV treated sample. Using Linear Interpolation Method, the IC25 (inhibition concentration that will cause a 25% reduction in survival or reproduction of the test organisms) was determined to be 25.0% effluent for the UV treated sample.

	Total Iron:	**Total Manganese:**
Sample 1:	**BDL**	**14 mg/L**
Sample 2:	**BDL**	**13 mg/L**
Sample 3:	**BDL**	**14 mg/L**

ESC Sample #: L708706-01 thru -13

Additional Toxicity Test Information

1). Submit copies of all bench sheets and statistical calculations/printouts obtained during the test(s). Data must be presented in tabular form and must include all physical and/or chemical measurements recorded during the tests (e.g. temperature, conductivity, total residual chlorine, dissolved oxygen, etc).

2). Methods/Instrumentation used in chemical analysis:

Dissolved Oxygen: YSI 5000 DO Meter/Probe (serial #01L0435)
pH: Beckman 390pH/Temp/mV/ISE Meter
Conductivity: Thermo Orion Model 150A+
pH/RDO/Conductivity: Thermo Scientific Orion VersaStar (serial #V 02105)
Water Bath: Lindberg/Blue, Model WB1140A-1 (serial #S01M-580360-SM)
Temperature: Thermometers calibrated to NIST certified thermometer
Alkalinity: Lachat
Hardness: Lachat
Total Residual Chlorine: Hach Pocket Colorimeter, Model #46770-00 (serial #971000112186)
Environmental Chambers: 25 degrees C + 1.0 degree - Thermo-Kool
Environmental Chambers (for Colorado tests): 20 deg C ± 1.0 degree - Thermo Scientific Model 3759
Light Quality: Ambient Lab Illumination
Light Intensity: 50-100 ft-c - SPER Scientific Light Meter 840021/Universal Enterprises Model DLM2
Photoperiod: 16 hours light, 8 hours dark
Drying: Overnight at greater than 60 deg Celsius in a Fisher Scientific Isotemp Oven, Model 655F
Mean Dry Weight: Determined using Mettler Toledo Balance, AT261 Delta Range
Reference Weights (Set #1): Class 1, TREOMNER, Inc., serial number 85035
Reference Weights (Set #2): Class 1, TREOMNER, Inc., serial number 67812

This method is performed only by Assistant Biologists, Biologists, and Senior Biologists that have laboratory personnel that are not experienced with toxicity testing will not handle test organisms during a toxicity evaluation. Lab Techs, Chemists, and others may assist (under supervision) with the gathering of data during the evaluation (pH, DO, conductivity, alkalinity, hardness, etc.), but will not be allowed to do any work with the test organisms themselves. The following analysts have met Technical Training Qualifications and their initials (in parenthesis) can be found on the bench sheets in this report: **Brandon Etheridge (BE); Shain W. Schmitt (SWS); Will Methvin (WM); Bridget Miller (BBM); Stacy Kennedy(SK); John Ariazi (JA); Becky Rush (BR)**

3). Indicate below any other relevant information that may aid in the evaluation of this report. Include any deviations from EPA methodology that were necessary for these tests as well as any sample manipulations which were performed, such as aeration, dechlorination with sodium thiosulfate, etc. and the justification for such manipulations or deviations. Attach additional pages as needed.

Toxicity was demonstrated during the prior evaluatuation (June 24- July1, 2014), therefore remaining effluent from the event was composited and treated via TIE manipulations to determine the extent of inhibition and look for increase/decrease in toxicity in response to each of the treatments. Datasheets are attached in the APPENDIX of this report outlining the procedures and detailing the survival and reproduction responses to each treatment.

ESC
L·A·B S·C·I·E·N·C·E·S

Facility/Discharger: Compliance Eng - Cothron's Grocery
TIE Lab Identification #: L708706-01 thru -13
TIE Start Date: 7-9-14

TIE Checklist

Yes or (No) Is the Baseline sample freshly collected (specifically for this TIE)?

If no, what is the original L# of the sample? L706249-01,02,03

How much volume is available for TIE from Sample #1? 2 gallons mL
How much volume is available for TIE from Sample #2? 2 gallons mL
How much volume is available for TIE from Sample #3? 2 gallons mL

Describe how Baseline sample was composited from the samples above (what samples were used? at what volumes?): Equal parts from each sample were used.

Example: 1,000mL from sample #1, 1,000mL from sample #2, and 1,000mL from sample #3 were add together to get a composite of with a total volume 3,000mL per container.

(C.dubia) Minnows What test species will be used? Duration of TIE? 24-hrs 48-hrs 96 hrs (chronic)

What TIE manipulations were chosen?

Total Iron = FEICP
Total Manganese = MNICP
Baseline
EDTA 3mg/L
EDTA 8mg/L
STS 10mg/L
STS 25mg/L
Aeration
GAC
pH3 Adj/Filt
pH11 Adj/Filt
Zeolite
Filtration
C-18
UV

Why were these manipulations chosen?

Full Spectrum
Client Request

Baseline Sample

Chlorine (prior to manipulation): <0.2 mg/L

Ammonia (prior to manipulation): <1 ppm

Notes regarding chlorine level after manipulations:

Chlorine (**post-Aeration**) NA mg/L
Other: NA mg/L

Make notes regarding ammonia level after manipulations:

Ammonia (**post-Aeration**) NA ppm
Ammonia (**post-Zeolite**) NA ppm

Does Baseline contain solids? Were solids removed by any manipulations?

No solids

Does Baseline contain odor? Was odor removed by any manipulations?

No Odor

What is general color of Baseline? Did color change with manipulation?

very clear
pH11 - color was dark Brown @ pH11 but was removed by filtering sample. BE 7-9-14

~~pH11~~
BE 7-9-14

Other comments:

pH3 turned slighly brown when NaOH (Base) was add to get pH up to inital pH.

Toxicity Identification Evaluation

Client Name Compliance Eng - Cothron's

ESC Sample # _____

Manipulation: pH Adjustment and Filtration

Sample Description	Amount Adjusted	Initial pH	Adjusted to	Amount HCl (1N) or ___ N	Amount NaOH (1N) or ___ N	Final pH
control pH 11	1,500mL	8.08	11	2.48mL	2.85mL	8.07
Cothron's pH 11	1,500mL	7.91	11	3.80mL	6.70mL ~~10mL~~ BE	7.92
control pH 3	1,500mL	8.05	3	3.38mL	2.71mL	8.04
Cothron's pH 3	1,500mL	7.85	3	4.19mL	1.70mL	7.86

ESC LAB SCIENCES

12065 LEBANON RD.
MT. JULIET, TN 37122
(800) 767-5859
WWW.ENVSCI.COM

Compliance Eng. - Cothron's Grocery
Chronic C.dubia pH/DO/Specific Conductance Readings

Original Lab ID: L706249-01,-02,-03 **Newly Assigned TIE Lab ID:** _____

pH & DO initial readings

	Wed - July 9, 2014 Analyst: BRM Time: 1540		
	initial pH	initial DO	initial Spec Con
Control	8.0	8.4	213
Baseline - 100%	7.9	8.7	252
EDTA 3 mg/L	8.1	8.2	243
EDTA 8 mg/L	8.1	8.2	245
STS 10 mg/L	8.2	8.4	253
STS 25 mg/L	8.2	8.4	272
Aeration CONTROL	8.1	8.5	221
Aeration - 100%	8.3	8.6	250
Control - GAC	9.6	8.6	196
GAC - 100%	9.5	8.1	184
Control (pH 3 Adj/Filt)	8.6	8.4	479
pH 3 Adj/Filt - 100%	7.8	8.4	472
Control (pH 11 Adj/Filt)	7.9	8.3	458
pH 11 Adj/Filt - 100%	8.0	8.0	597
Control - Zeolite	8.0	7.9	219
Zeolite - 100%	8.2	8.3	246
Control - Filtration	8.2	8.3	219
Filtration - 100%	8.0	8.1	258
Control (C-18 Column)	8.1	8.3	216
C-18 Column - 100%	8.2	8.4	255
Control - UV Treatment	8.1	8.4	214
UV treatment - 100%	7.9	8.4	249

(final readings) pH & DO after 24-hrs

	Thur - July 10, 2014 Analyst: JA Time: 10:54	
	final pH	final DO
Control	7.9	8.4
Baseline - 100%	8.3	8.3
EDTA 3 mg/L	8.3	8.4
EDTA 8 mg/L	8.3	8.4
STS 10 mg/L	8.3	8.3
STS 25 mg/L	8.3	8.3
Aeration CONTROL	7.9	8.5
Aeration - 100%	8.2	8.3
Control - GAC	8.3	8.1
GAC - 100%	8.3	8.1
Control (pH 3 Adj/Filt)	7.3	8.0
pH 3 Adj/Filt - 100%	7.0	8.1
Control (pH 11 Adj/Filt)	8.0	8.2
pH 11 Adj/Filt - 100%	8.2	8.2
Control - Zeolite	8.0	8.0
Zeolite - 100%	8.2	8.1
Control - Filtration	8.1	8.1
Filtration - 100%	8.2	8.2
Control (C-18 Column)	8.1	8.2
C-18 Column - 100%	8.2	8.1
Control - UV Treatment	8.1	7.9
UV treatment - 100%	8.2	7.9

Notes: TIE tests to be run with *Ceriodaphnia dubia* only.